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STUDIES ON POLLEN IRRADIATION IN RICE (*ORYZA SATIVA* LINN.)

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ABSTRACT

Pollen stainability after irradiation at 1-9 Kr increased with radiation dose. A low frequency of anaphase bridges and fragments were found in mitotic cells of M_2 plants. In general, the frequency of chlorophyll seedling mutations increased as gamma radiation dose increased with a peak at 6-7 Kr. Likewise, mutant types like short plant and medium-sized grains found at the dose of 7 Kr were selected from the M_2 generation for possible progeny testing.

Introduction

Most mutation breeding studies utilized dormant seeds as materials for irradiation studies, a method known to be largely affected by diplontic selection. One way of avoiding this problem is irradiation of pollen grains which, when done in combination with hybridization, could increase variability and widen the basis for selection. Thus, pollen grains are probably more suited for mutational studies than seeds since the pollen is a relatively simple, haploid radiation target which have been shown to withstand a variety of extreme experimental conditions without impairing their function of transporting and delivering the male genetic contribution to the egg (Pandey, 1983).

In addition, if genes expressed in the endosperm were analyzed, their mutation or loss is easily detected in the M_1 generation by the use of treated pollen carrying dominant genes (Gavazzi, 1983). Moreover, the M_1 material after pollen irradiation is much easier to handle than after seed irradiation because the occurrence of chimeras in M_1 plants is avoided as the irradiated pollen is applied at the flowering stage.

The main problem in pollen irradiation, however, lies in the difficulty of obtaining sufficient materials in some species and the rather brief viability of pollen grains. These disadvantages could be overcome, however, by proper handling techniques as the pollen of some species could be kept viable for several months. Large quantities of pollen could be obtained from most naturally cross-pollinated plants.

The objectives of this study were: (1) To determine the effects of varying doses of gamma radiation on pollen stainability and M_1 seed set; (2) To study the types and frequency of chromosomal aberrations in the mitotic stages; (3) To determine the mutation frequency in the M_2 generation following pollen irradiation; and (4) To identify treatments that yield high mutation rates.

Materials and Methods

This study was conducted at the Botany Experimental Garden, Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City using seeds from a pure line of Azucena variety of rice, *Oryza sativa* Linn., obtained at the Philippine Atomic Research Center (now, The Philippine Nuclear Research Institute) in Diliman, Quezon City.

About 200 seeds, with a 14% moisture content, for each treatment were sown on moist filter paper in petri dishes following a staggered time schedule of two weeks interval for each treatment. After 14 days the seedlings were transplanted in rice paddies measuring about 5 m x 10 m at the rate of one seedling per hill at distances of 40 cm between rows and 30 cm in the row. These plants served as parent materials. The ovule parents were emasculated using the combined technique of the hot-water method (Chandraratna, 1964; Figure 1) and the clip method (Coffman and Herrera, 1980).

Figure 1. Set up for hot-water emasculation method.

After emasculation, a glassine bag was immediately placed over the panicle (Figure 2). The same procedure was applied to the ovule parent which served as control.

Pollen stainability. At least 25 blooming panicles were used as male parent for each treatment-replication including the control. The panicles were collected early in the morning, wrapped in moist cheese cloth and brought to the Philippine

Figure 2. Rice panicles covered with glassine bags.

Nuclear Research Institute for irradiation at doses of 1, 3, 5, 7 and 9 Kr gamma rays in the Cobalt-60 facility of the Philippine Nuclear Research Institute, Diliman, Quezon City. Unirradiated blooming panicles served as pollen control.

Within 12 hours after pollen irradiation, a random sample of 100 pollen grains from each treatment were tested and scored under the light microscope for pollen stainability using Alexanders' stain technique (Alexander, 1969). The rest of the pollen were used for pollinating the emasculated panicles which served as the ovule parent.

To apply the treated pollen, the glassine bags covering the emasculated panicles were removed. Then the irradiated pollen grains were dusted on the stigmas of the emasculated florets. The glassine bags were quickly placed back over the pollinated panicles. The ovule parent plants were grown to maturity. At harvest, the M_1 grains were collected separately for data on seed set.

M_1 Planting. A sample of 100 M_1 rice seeds was selected at random from each treatment-replication for weight determination. A random sample of 25 M_1 seeds from each treatment-replication was sown on moist filter paper in petri dish for gathering data on seed germination and for cytological examination of root tips in the M_1. The root tips were examined for occurrence of chromosomal aberrations at anaphase and telophase stages using Khans' staining technique for rice chromosomes (Khan, 1975).

For each treatment-replication, 200 M_1 seeds of the pollinated panicles were sown in seed boxes filled with sterilized garden soil in a randomized complete block design replicated three times. Only one seed was sown per hill. Thirty seedlings taken at random from each treatment-replication were used for gathering data on seedling height 12 days from sowing. These M_1 seedlings were then transplanted in the field in a dose to row plan randomized with three replications at the rate of one seedling per hill at distances of 40 cm between rows and 30 cm in the row. These M_1 plants were grown to maturity for data on days to flowering, number of grain-bearing tillers, mature plant height, length of panicles, types and frequency of

M_1 morphological characters and M_2 pollen stainability. The primary tillers of the M_1 plants were bagged to insure self-pollination. Seeds from the bagged panicles were then harvested separately for data on M_2 seed set.

M_2 Planting. From a random sample of 10 lines of M_1 plants from each treatment, 100 M_2 seeds from each treatment-replication were sown on moist filter paper in petri dish for seed germination in the M_2. Twenty-five germinating seeds were collected for cytological examination of the root tips in the M_2. The rest of the seeds from each M_1 plant were sown in seed boxes for data on chlorophyll seedling mutations.

The chlorophyll mutation spectrum was separated into viable and lethal types.

A random sample of 25 plants from the M_1 lines and 33 seedlings from each treatment-replication were transplanted in the field in a dose-to-row-plan randomized with three replications at the rate of one seedling per hill at distances of 20 cm between rows and 15 cm in the row. As in the M_1 planting, these M_2 plants were grown to maturity for data on days to flowering, number of grain-bearing tillers, mature plant height, length of panicles and on possible mutant M_2 characters.

Results and Observations

1. *M_1 Pollen stainability.* Table 1, Column 2 indicates that there was a reduction in pollen stainability with increasing doses of gamma radiation. Reduction in pollen stainability was already significant after the 1 Kr with 73.25% which correspond to 95.34% of the percentage of control (Figure 3). Highly significant differences in pollen stainability were observed among the treatments: control and 1 Kr, 3 and 5 Kr, 5 and 7 Kr and 7 and 9 Kr. No significant difference was noted between doses of 1 and 3 Kr gamma rays. Stainable pollen grains were whole, round grains which gave a deep red to dark purplish color reaction to Alexander's stain (Alexander, 1969) while the non-stainable pollen grains were deformed, shrivelled grains and gave a greenish color reaction to Alexander's stain (Figure 4).

2. *M_1 Seed set.* Low mean values for percent seed set was noted among the treatments. The mean percentage seed set decreased significantly and linearly with increasing radiation dose at 1, 3 and 9 Kr gamma rays (Table 1, Column 3). Significant reduction in percent seed set was evident after 1 Kr with 48.70% which correspond to 90.96% of the percentage of control (Figure 3). Doses of 3, 5 and 7 Kr gamma rays with 46.55%, 45.80% and 45.39% respectively, were not significantly different with each other.

3. *M_1 Seed weight.* The effects of the different treatments on M_1 seed weights are presented in Table 1, Column 4. Mean seed weight was noted to decrease significantly after 1 Kr gamma rays with 2.25 gms. Figure 5 shows that there was a slight increase in mean seed weights at 3 Kr with 90.91% and at 7 Kr with 76.28%. Treatments 1 and 3 Kr as well as between 5 and 7 Kr were not significantly different (Table 1, Column 4).

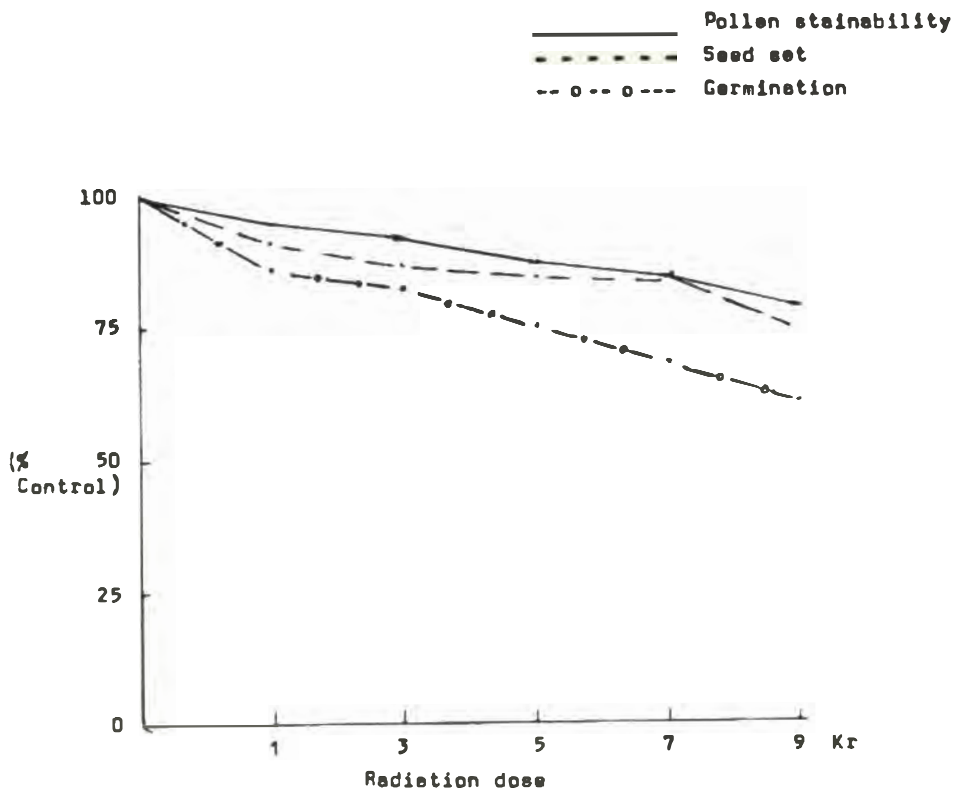

Figure 3. Mean percentages of pollen stainability, percent seed set and seed germination of M_1 plants after pollen irradiation.

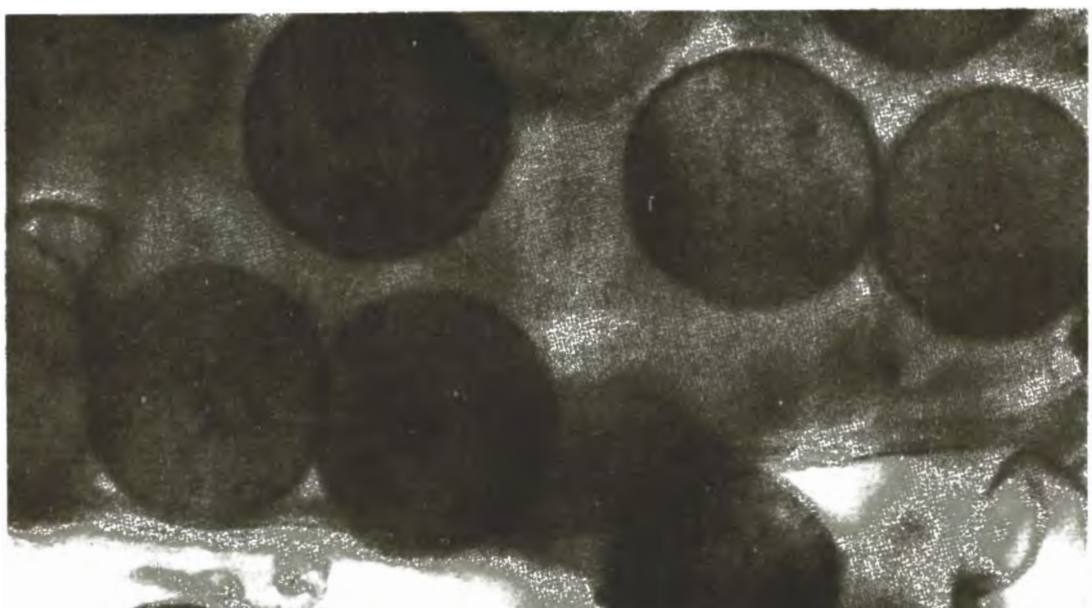

Figure 4. Stainable and non-stainable rice pollen grains. X 80.

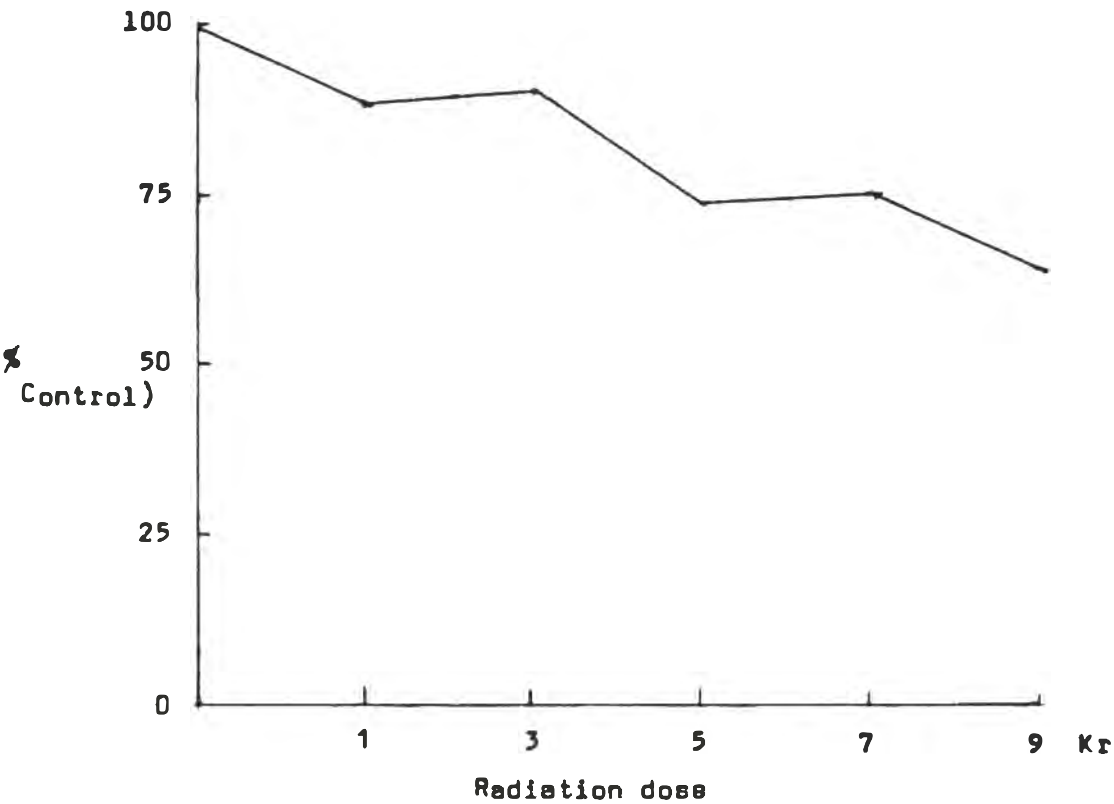

Figure 5. Mean weights of 100 M_1 rice seeds selected at random after pollen irradiation.

4. *M_1 Germination percentage.* Highly significant differences in germination were observed among the treatments. Table 1, Column 5 shows that mean seed germination percentages were reduced in direct proportion to pollen irradiation and only 60.85% of the control germinated at the highest dose, 9 Kr (Figure 3). No significant differences were noted among 1 and 3 Kr, 3 and 5 Kr, 5 and 7 Kr and 7 and 9 Kr.

5. *M_1 Seedling height.* Table 1, Column 6 summarizes the mean seedling heights 12 days after sowing. Highly significant differences were noted among the treatments: control and 5 Kr, control and 7 Kr, control and 9 Kr, 3 and 5 Kr, 7 and 9 Kr. Significant reduction in seedling height was noted after 5 Kr gamma rays with 8.71 cm and 72.89% as compared with the control (Figure 6 and 7). LD_{50} was found to be approximately 8.30 Kr gamma rays (Figure 6). A stimulatory effect of pollen irradiation on seedling height was noted after the 3 Kr treatment. Although there was a slight increase in seedling height after the 3 Kr, there was no significant difference between 1 and 3 Kr pollen exposures. The following treatments were not significantly different with each other: control, 1 and 3 Kr, 1 and 5 Kr, and 5 and 7 Kr.

6. *M_1 Chromosomal abberation.* There were only two types of aberrations obtained i.e. bridges and fragments (Figures 8, 9, and 10). The most frequently observed aberrations were anaphase bridges.

Table 1. Means of percentages of pollen stainability, seed set, seed weights (gm), seed germination, seedling heights (cm) and chromosomal aberration of M_1 rice plants after pollen irradiation

Gamma ray dose (Kr)	*M_1 Pollen Stainability (%)*	*M_1 Percent Seed set (%)*	*M_1 Seed Weight (g)*	*M_1 Seed Germination (%)*	*M_1 Seedling Height (cm)*	*M_1 Chromosomal Aberrations (%)*
0	76.83^{a1}	53.54^{a1}	2.53^{a1}	86.15^{a1}	11.95^{a1}	0^{c1}
1	73.25^{b}	48.70^{b}	2.25^{b}	75.20^{ba}	10.80^{b}	20.18^{b}
3	71.84^{b}	46.55^{c}	2.30^{b}	71.19^{bc}	11.10^{a}	23.48^{b}
5	67.84^{c}	45.80^{c}	1.90^{c}	65.89^{cd}	8.71^{bc}	24.61^{b}
7	65.61^{d}	45.39^{c}	1.93^{c}	59.81^{de}	7.92^{c}	23.99^{b}
9	61.42^{e}	40.35^{d}	1.68^{d}	52.42^{e}	5.12^{d}40.50	40.50^{a}

[1] Means having a common letter(s) are not significantly different at 5% level of significance (DMRT).

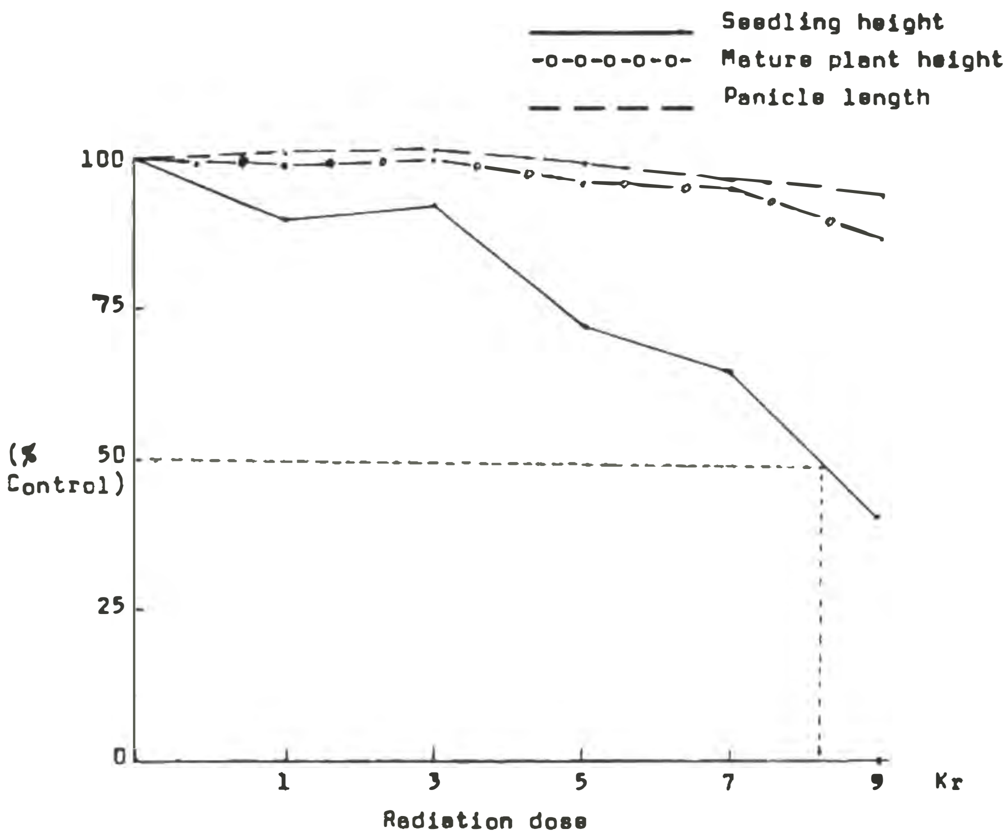

Figure 6. Mean seedling height, mature plant height and panicle length of M_1 rice plants after pollen irradiation.

Figure 7. Seedling height of M_1 rice plants after pollen irradiation.

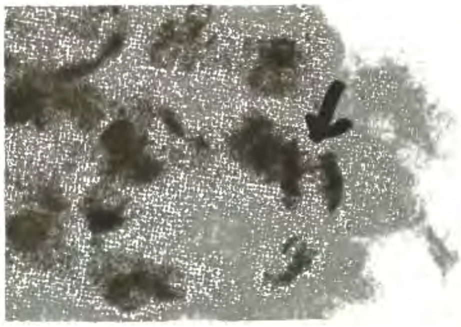

Figure 8. Telophase stage with bridge X 320.

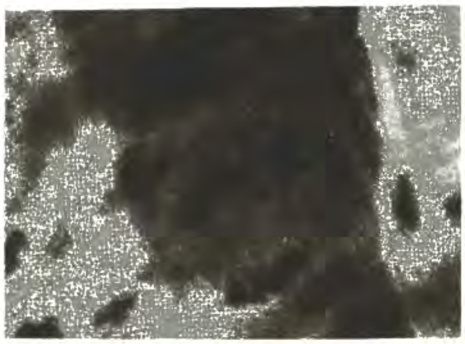

Figure 9. Telophase stage with fragment X 320.

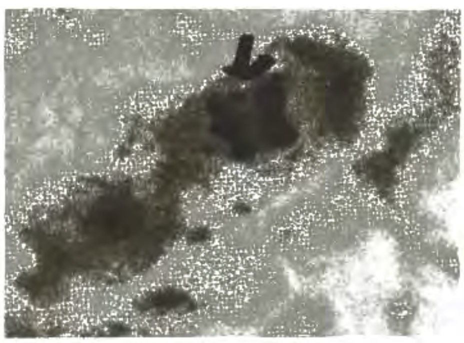

Figure 10. Telophase stage with fragment X 320.

Table 1, Column 7 summarizes the mean percentages of chromosomal aberrations in mitotic root tips of M_1 rice seeds. The mean percentages of chromosomal aberrations of 0.00%, 20.18%, 23.48%, 24.61% and 40.50% increased with increasing levels of radiation except at 7 Kr, in which there was a slight decrease (Figure 11). The highest percentage of aberration was noted after the 9 Kr which gave 40.50%. No significant differences were noted among doses of 1 and 3 Kr, 3 and 5 Kr, 5 and 7 Kr.

7. *M_1 Number of days to flowering.* The mean number of days to flowering ranged from 88 to 91 (Table 2, Column 2). There were no significant differences among the treatments.

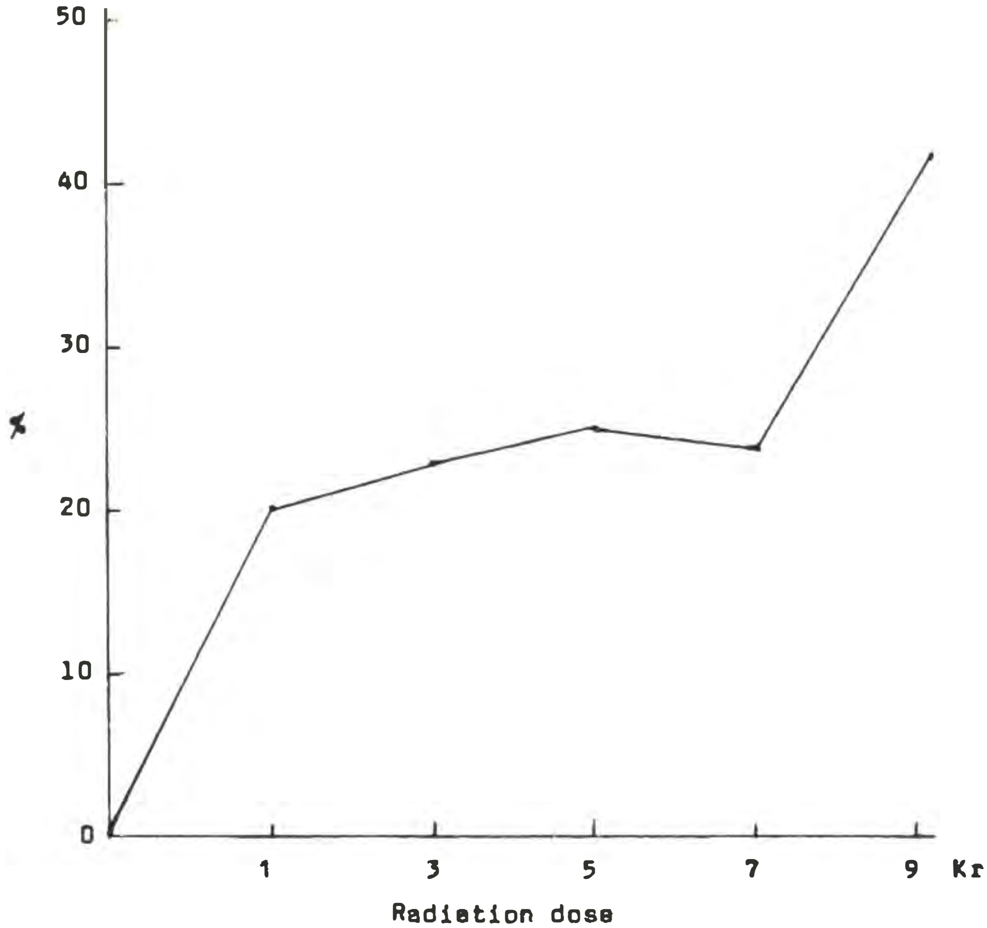

Figure 11. Mean percentages of chromosomal aberrations in mitotic root tips of M_1 rice seeds after pollen irradiation.

8. *M_1 Number of grain-bearing tillers.* The effects of the different treatments on M_1 number of grain-bearing tillers are presented in Table 2, Column 3. The average number of grain-bearing tillers ranges from 15.33 to 17.33. It can be seen that all the treatments influenced the number of grain-bearing tillers formed except the pollen exposure at 9 Kr which had no effect. The greatest increase in the number of grain-bearing tillers occurred after the 3 Kr treatment (Figure 12). Also at 3 Kr a stimulatory effect of pollen irradiation was greatly expressed.

9. *M_1 Mature plant height.* Heights of mature M_1 plants are shown in Table 2, Column 4. A slight insignificant increase on mature plant height was noted after

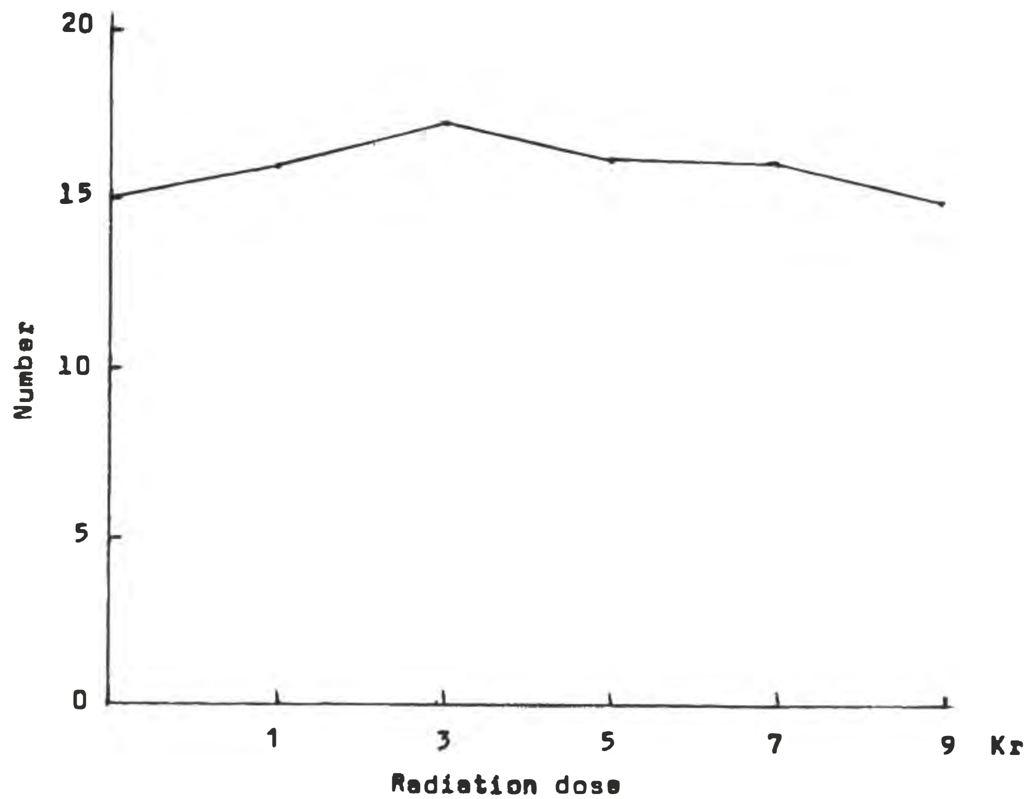

Figure 12. Mean number of grain-bearing tillers in M_1 rice plants after pollen irradiation.

3 Kr as compared with the control (Figure 6). No significant differences were noted among 1, 3, 5 and 7 Kr gamma ray doses.

10. *M_1 Panicle length.* Table 2, Column 5 summarizes the mean panicle length (cm) of the primary tillers of M_1 plants. A stimulatory effect of pollen irradiation was noted after 1 and 3 Kr with 37.15 and 37.14 cm respectively, as compared with 36.52 cm of control. A significant reduction was observed after the 7 and 9 Kr, however, the decrease in panicle length after the 7 Kr was not significant as compared with the control (Figure 6).

11. *M_1 Morphological characters.* The types, frequencies and description of morphological characters are presented in Table 3.

The morphological characters observed were on grain sizes, i.e. short, broad grains (Figure 13); short, narrow, fine grains (Figure 14); long, narrow, fine grains; long broad, coarse grains; short, small, fine grains; bushy habit, drooping leaves; high tillers plants; semi-sterile and completely sterile plants; short and narrow tillers with short, fine leaves.

12. *M_2 Pollen stainability.* Table 4, Column 2 shows that mean percentages of pollen stainability were reduced in direct proportion to pollen irradiation. A significant reduction in M_2 pollen stainability was noted after 5 Kr which gave 62.68%

Figure 13. Rice grains: A. Control – long grains, X 0.55 mm; B. Mutant – short, broad grains, X 0.846 mm.

Figure 14. Rice grains: A. Control – long grains, X 0.917 mm; B. Mutant – short, narrow, fine grains, X 0.888 mm.

which corresponds to 81.73% of the control (Figure 15). No significant differences between the control, 1 and 3 Kr; and between doses 5, 7 and 9 Kr.

13. *M_2 Percent seed set.* The mean percent M_2 seed set ranged from 31.97% to 50.27% (Table 4, Column 3). There were no significant differences among the treatments.

14. *M_2 Germination percentage.* Table 4, Column 4 summarizes the mean germination percentage 7 days after sowing. A significant reduction in M_2 germination percentage was evident after 7 Kr gamma rays with 69.83% which corresponds to 91.00% of the control (Figure 15).

15. *M_2 Chromosomal aberrations.* The most frequently observed aberrations were anaphase bridges.

Table 2. Means of days to flowering, number of grain-bearing tillers, mature plant heights (cm) and panicle lengths (cm) in M_1 rice plants after pollen irradiation

Gamma ray dose (Kr)	*M_1 Days to Flowering*	*M_1 Number of Grain-bearing Tillers*	*M_1 Mature Plant Height (cm)*	*M_1 Panicle Length (cm)*
0	89	15.33^{b}[1]	166.58^{a}[1]	3652^{ab}[1]
1	88	15.67^{b}	164.45^{a}	37.15^{a}
3	90	17.33^{a}	166.76^{a}	37.14^{a}
5	89	16.00^{b}	160.70^{a}	36.46^{ac}
7	89	16.00^{b}	160.27^{a}	35.48^{bcd}
9	91	15.33^{b}	147.96^{b}	34.88^{d}

[1] Means having a common letter(s) are not significantly different at 5% level of significance (DMRT).

Table 3. Types of morphological characters observed in M_1 rice plants after pollen irradiation

Gamma ray dose (Kr)	*No. of plants*	*Characteristics of Variants*
0	0	None
1	2	Short, narrow tillers, short, fine leaves.
	1	Tall, high tillering capacity (28 tillers as compared with control with maximum number of 26).
3	2	Short, narrow tillers, short, fine leaves.
	5	Tall, high tillering capacity (29 tillers).
5	2	Short, narrow tillers, short, fine leaves.
	1	Tall, bushy habit, dropping leaves, high tillering capacity (32 tillers).
	1	Tall, high tillering capacity (27 tillers).
	1	Tall, long, narrow, fine M_2 grains.
	1	Tall, long, broad, coarse M_2 grains.
7	2	Tall, completely sterile plants.
	4	Tall, semi-sterile, long narrow, fine M_2 grains.
	1	Very short, narrow tillers, short, fine leaves.
	4	Tall, high tillering capacity (28-31 tillers).
	1	Tall, semi-sterile, short, broad M_2 grains.
	2	Tall, semi-sterile, short, narrow, fine M_2 grains.
9	4	Short, narrow tillers, fine leaves.
	1	Tall, completely sterile plant.
	1	Tall, high tillering capacity (27 tillers).
	1	Tall, semi-sterile (probably approx. 5% gran set), long, big M_2 grains.
	1	Tall, semi-sterile, long, narrow, fineM_2 grains.
	1	Tall, almost completely sterile (probably only 3% grain set), short, small, fine M_2 grains.
	1	Tall, semi-sterile, short, narrow, fine M_2 grains.
	1	Tall, semi-sterile, short, broad M_2 grains.

Table 4. Means of percentages of pollen stainability, seed set, seed germination and chromosomal aberration of M_2 rice plants after pollen irradiation

Gamma ray dose (Kr)	*M_2 Stainability Stainability (%)*	*M_2 Percent Seed set (%)*	*M_2 Seed Germination (%)*	*M_2 Chromosomal Aberrations (%)*
0	76.69^{a}[1]	48.97	76.73^{a}[1]	0 ^{b}[1]
1	75.34^{a}	46.09	76.13^{a}	20.75^{a}
3	70.66^{a}	50.27	76.66^{a}	21.76^{a}
5	62.68^{b}	43.62	79.66^{a}	22.60^{a}
7	57.80^{b}	44.92	69.83^{b}	20.73^{a}
9	54.87^{b}	31.97	65.00^{b}	25.37^{a}

[1]Means having a common letter(s) are not significantly different at 5% level of significance (DMRT).

Like in the M_1, the mean percentages of chromosomal aberration in the M_2 also increased with increasing levels of radiation except at 7 Kr with 20.73% which represent an insignificant reduction (Table 4, Column 5; Figure 16).

16. *M_2 Number of days to flowering.* The mean number of days to flowering ranged from 96 to 102 days (Table 5, Column 2). There were no significant differences among the treatments.

17. *M_2 Number of grain-bearing tillers.* Table 5, Column 3 shows that there were no significant differences among the treatments. The mean number of grain-bearing tillers ranged from 3.67 to 4.67.

18. *M_2 Mature plant height.* The mean heights of M_2 mature plants are shown in Table 5, Column 4. There were no significant differences among the treatments.

Table 5. Means of days to flowering, number of grain-bearing tillers, mature plant heights (cm) in M_2 rice plants after pollen irradiation

Gamma ray Dose (Kr)	*M_2 Days to Flowering*	*M_2 Number of Grain-bearing Tillers*	*M_2 Mature Plant Height (cm)*	*M_2 Panicle Length (cm)*
0	100	4.67	125.55	30.14^{a}[1]
1	102	3.67	118.42	28.60^{bc}
3	102	3.67	114.09	27.86^{c}
5	101	3.67	111.79	29.09^{b}
7	96	4.67	117.17	27.94^{c}
9	96	4.00	122.04	28.54^{bc}

[1]Means having a common letter(s) are not significantly different at 5% level of significance (DMRT).

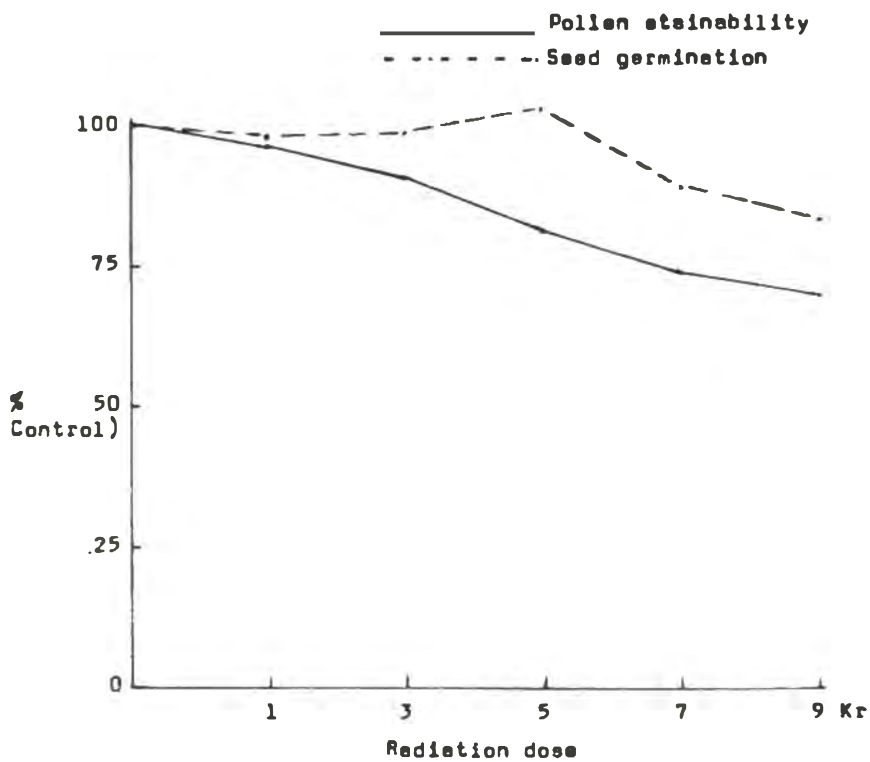

Figure 15. Mean percentages of pollen stainability and seed germination of M_2 rice plants after pollen irradiation.

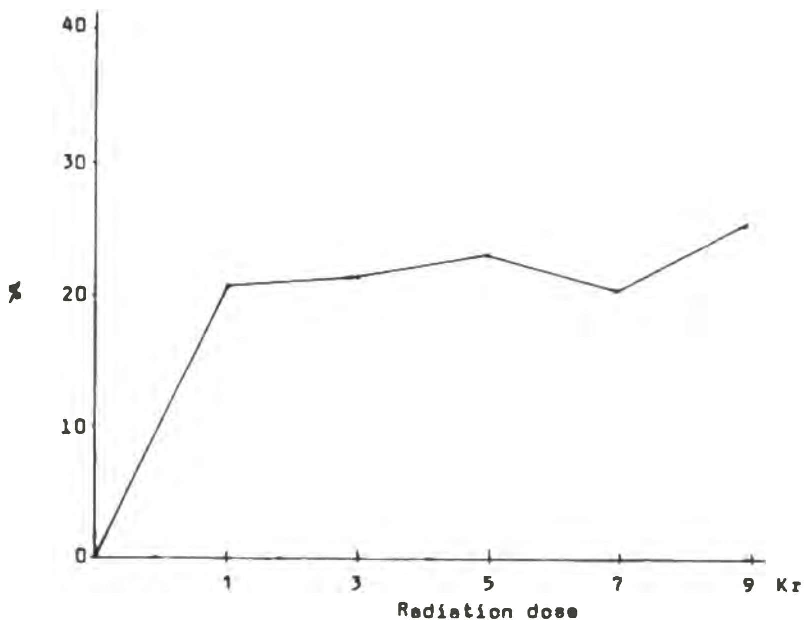

Figure 16. Mean percentages of chromosomal aberrations in mitotic root tips of M_2 rice seeds after pollen irradiation.

19. *M_2 Panicle length.* Highly significant differences in mean panicle lengths were observed among the treatments (Table 5, Column 5). A significant reduction in mean M_2 panicle length was noted at 1 Kr with 28.60 cm which corresponds to 94.89% of the control (Figure 17).

20. *M_2 Seedling mutation frequency.* Tables 6 and 7 show the chlorophyll mutation frequencies.

Based on the M_1 plants and M_2 seedlings, the 1 Kr pollen exposure had lower chlorophyll mutation rates with 4.19% for the former and 0.34% for the latter, while the 7 Kr treatment had the highest with 15.11% and 1.72%, respectively. A decrease in chlorophyll mutation rate was observed after the 9 Kr.

Based on leaf color, the chlorophyll mutation types obtained were *albina*, a lethal mutation characterized by entirely white leaves of the seedlings (Figure 18); *chlorina*, characterized by persistent yellow-green leaves; usually a viable mutation (Figure 19); *virescent*, characterized by pale green color of the leaves; a viable mutation (Figure 20); and *striata*, characterized by having leaves with longitudinal stripes of white on green or yellow on green (Figures 21 and 22).

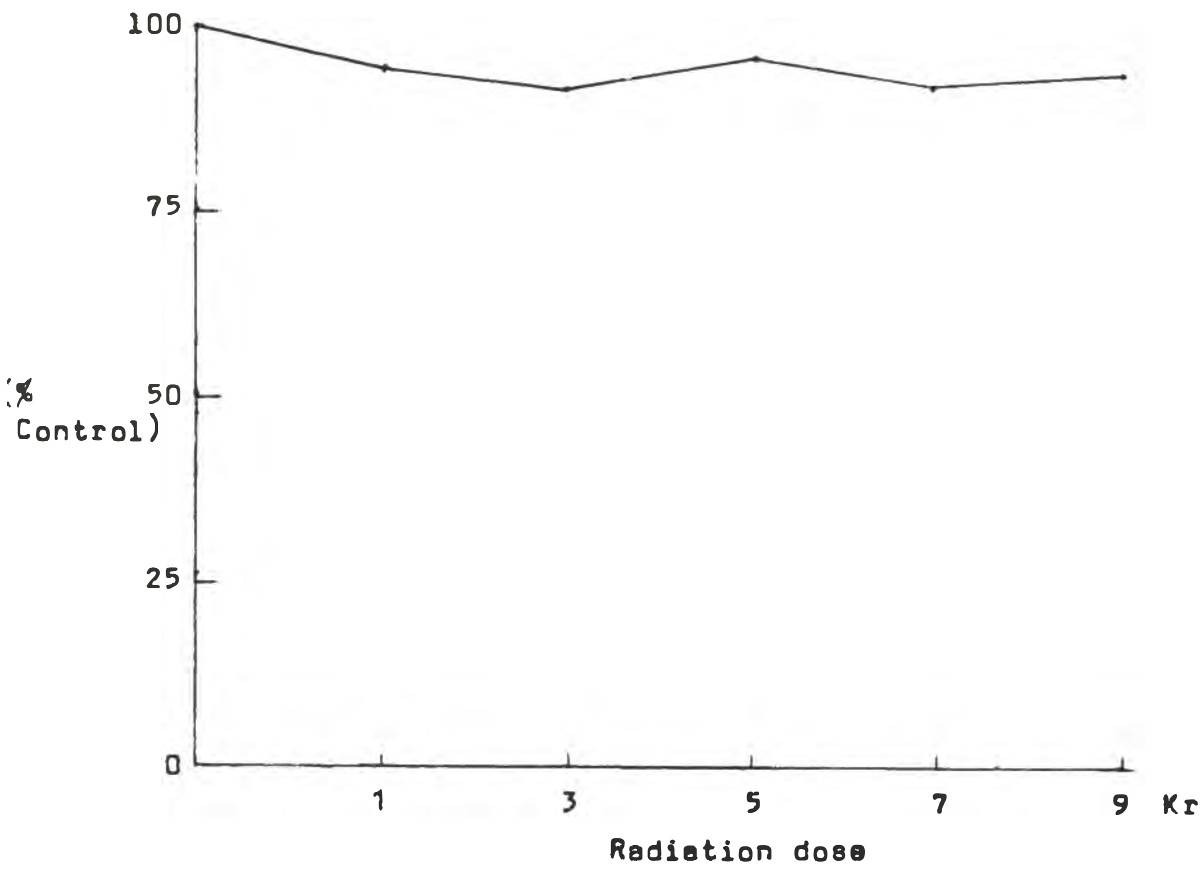

Figure 17. Mean panicle length (cm) of M_2 rice plants after pollen irradiation.

Table 6. Frequency of chlorophyll mutations (M_1 and M_2) induced in rice plants after pollen irradiation

Gamma ray dose (Kr)	*No. Studied*		*No. of M_1 Plants Segregating*	*No. of M_2 Mutants Seedlings*	*% Chlrophyll Mutation*	
	M_1 Plants	*M_2 Seedlings*			*M1 Plants*	*M_2 Plants*
0	75	11,860	0	0	0	0
1	215	28,557	9	98	4.19	0.34
3	189	22,461	11	192	5.82	0.85
5	172	19,786	18	227	10.46	1.15
7	139	16,643	21	286	15.11	1.72
9	121	13,164	13	123	10.74	0.93

Table 7. Frequency of chlorphyll mutation types in M_2 rice seedlings after pollen irradiation

Gamma ray dose (Kr)	*Total M_2 Seedlings*	*Types of Seedling Mutations*							
		Albina		*Chlorina*		*Virescent*		*Striata*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
0	11,860	0	0	0	0	0	0	0	0
1	28,557	26	0.09	26	0.09	46	0.16	0	0
3	22,461	63	0.28	0	0	129	0.57	0	0
5	19,786	19	0.10	0	0	184	0.93	24	0.12
7	16,643	69	0.41	0	0	211	1.27	6	0.14
9	13,164	11	0.08	2	0.02	108	0.82	2	0.02

Figure 18. Albina seedlings.

Figure 19. Chlorina seedlings.

Figure 20. Virescent seedlings.

Figure 21. Striata chlorophyll mutation.

Albina in the M_2 seedlings (Table 7) was noted to be highest after the 3 Kr with 0.28% and at 7 Kr with 0.41% and at its lowest after the 9 Kr treatment with 0.08%. *Chlorina* were observed to be present only after pollen exposures of 1 Kr which gave the highest frequency of 0.09% and 9 Kr which gave the lowest frequency of 0.02%. *Virescent* appears to increase in frequency of 0.16%, 0.57%, 0.93% and 1.27% with increasing gamma ray doses but decreased markedly after the 9 Kr treatment. For *striata*, the 5 Kr treatment had the highest frequency of 0.12%, while the two higher treatments, 7 and 9 Kr had the lowest frequency of 0.04% and 0.02%, respectively.

Figure 22. Striata chlorophyll mutation.

Comparing the viable (chlorina, virescent and striata) and lethal (albina) types in the M_2, the viable chlorophyll mutations were pre-dominant (Table 8).

21. *M_2 Morphological and other mutations.* The frequencies of induced morphological and other mutations are shown in Table 9. Based on either the M_1 or M_2 plants, the highest percentage of mutations occurred at 7 Kr which gave 10.07% for M_1 plants and 12.57% for M_2 plants. Percent morphological and other mutations were noted to decrease afer 9 Kr in both M_1 and M_2 plants with 9.09% and 10.12%, respectively.

The description of the morphological and other mutants are presented in Table 10. The desirable mutations like medium height, early flowering appeared after the 7 Kr and 9 Kr treatments.

Discussion of Results

The results of this study indicate the possible occurrence of differences in the zygotic lethality (or viability) of the treated and untreated pollen grains. The ability of the treated grains to fertilize the female parent was observed to be lower in comparison to that of the untreated pollen considering the percentage seed set obtained in the M_1.

The failure and/or reduced seed set obtained in this study could be attributed to the failure of the zygote to develop which is due to the presence of dominant lethals in the gametes contributed by the treated pollen grains. This lethality prevents embryo development but does not alter the capacity of the pollen grain to participate in fertilization normally even in competition with untreated pollen. Evidences in support of this assumption comes from previous studies of several workers. Brown and Cave (1953; 1954) working with *Lilium* pollen reported that x-irradiation of such pollen with 2,000 to 4,000 r units of x-rays did not appear

Table 8. Mutation spectrum in M_2 rice seedlings after pollen irradiation

Gamma ray dose (Kr)	Total mutant Seedlings	Viable		Lethal	
		No.	%	No.	%
0	0	0	0	0	0
1	98	72	73.47	26	7.93
3	192	129	67.19	63	17.40
5	227	201	88.55	19	3.58
7	286	217	75.87	69	12.32
9	123	112	91.06	11	4.14

Table 9. Frequency of morphological and other mutations (M_1 and M_2) induced in rice plants after pollen irradiation

Gamma ray dose (Kr)	No. Studied		No. of M_1 Plant Segregating	No. M_2 Mutant Plants	Percent Mutation	
	M_1 Plants	M_2 Mature Plants			M_1 Plants	M_2 Plants
0	75	572	0	0	0	0
1	215	496	3	38	1.40	7.66
3	189	777	7	57	3.70	7.34
5	172	619	6	75	3.49	12.12
7	139	557	14	70	10.07	12.57
9	121	563	11	57	9.09	10.12

Table 10. Types of morphological mutations observed in M_2 rice plants after pollen irradiation

Gamma ray dose (Kr)	*No. of Plants*	*Characteristics of Mutants*
0	0	None
1	38	Short, narrow tillers, short, fine leaves
3	57	Short, narrow tillers, short, fine leaves (Fig. 23)
5	68	Short, narrow tillers, short, fine leaves.
	3	Short, broad leaves
	3	Very short, very late flowering (about 30 days late compared with control), narrow few tillers (2-3 tillers), short, fine leaves
	1	Short, very late flowering (131 days from sowing to heading, about 42 days late compared with control), narrow tillers, short, fine leaves
7	6	Medium height, early flowering (80-85 days from sowing to heading, about 5-10 days ahead of control)
	5	Tall, early flowering (80-85 days from sowing to heading, about 5-10 days ahead of control)
	1	Short, very late flowering (118 days from sowing to heading, about 29 days late compared with control), narrow tillers, short, fine leaves
	2	Medium height, with variegated leaf (yellowish green margin, white midvein) (Fig. 24)
	53	Short, narrow tillers, short, fine leaves
	2	Short, narrow tillers, short, fine leaves, sm1 ll, narrow fine M_3 grains
	1	Tall, high tillering capacity (20 tillers)
9	1	Short, with variegated leaf (yellow margins, white midvein and some lateral veins (Fig. 25)
	4	Tall, with variegated leaf (Fig. 26)
	2	Tall, almost completely sterile, short, narrow, fine M_3 grains
	4	Tall, semi-sterile (about 50% seed set)
	4	Medium height, semi-sterile
	31	Short, narrow tillers, short, fine leaves
	4	Very short, narrow tillers, short, small M_3 grains (Fig. 27)
	8	Early flowering (80-85 days from sowing to heading, about 5-10 days ahead of control), medium height.

Figure 24. Medium height M_2 plant with variegated leaf.

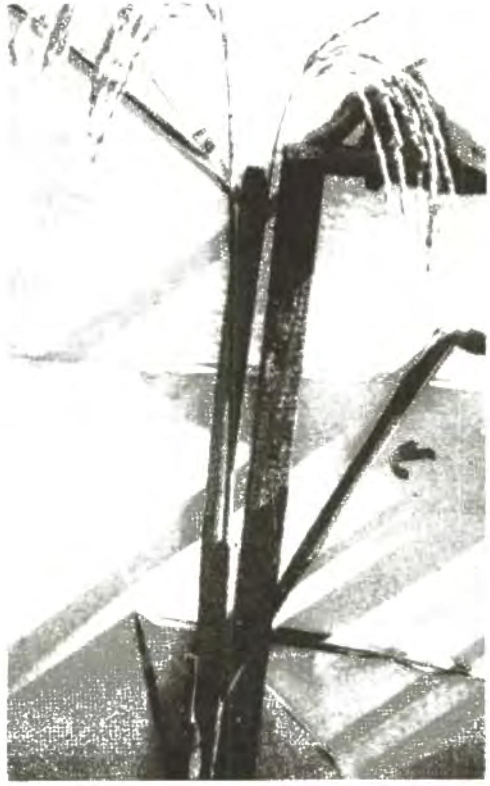

Figure 23. Short M_2 plant, narrow tillers, fine leaves.

Figure 25. Short M_2 plant with variegated leaf.

Figure 26. Tall M_2 plant with variegated leaf.

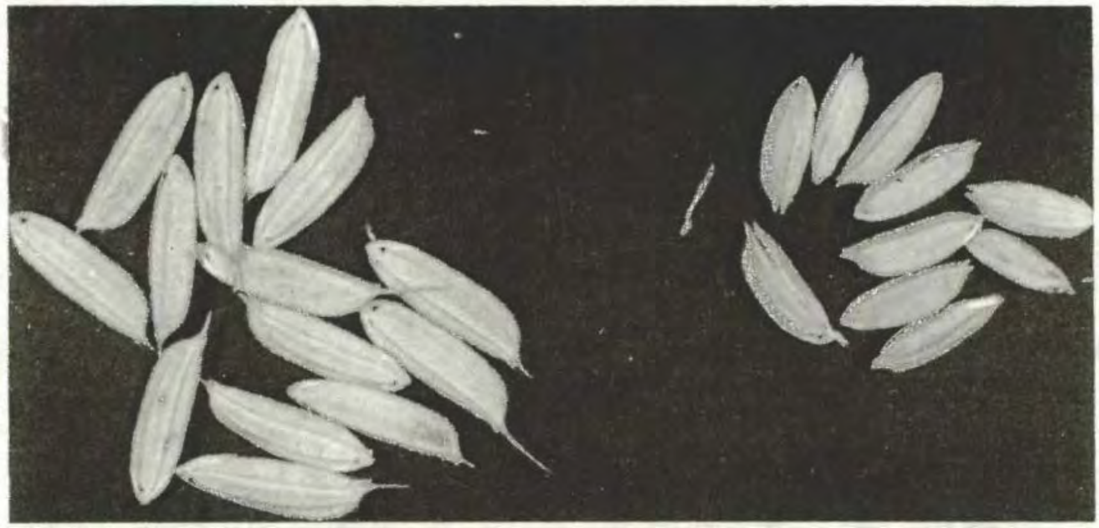

Figure 27. Rice grains: A. Control – long grains, X 0.687 mm; B. Mutant – short, small grains, X 0.666 mm.

to alter fertilization ability. However, the sharp decrease in the number of seeds produced suggested that after fertilization by irradiated pollen, the zygotes aborted, presumably because of the production of dominant lethals by the irradiation, rather than failure of the irradiated pollen grains to achieve fertilization. Donini and Hussain (1968) found no differences in the percentage of fertilization after irradiation of either the male or the female gamete of *Triticum durum.* In both cases there was a similar failure of zygote formation and proembryo growth.

The possibility that mechanical difficulties in mitoses are the most effective causes of the abnormalities indicating dominant lethality in *Lilium* is probably also operative in rice after pollen irradiation as is indicated by the presence of bridges, and fragmented chromosomes which could present problems of chromosome movement.

The fact that there is gradual reduction in percent seed set as radiation dosage is increased probably indicates that there is complete abortion at some stage in the development of most ovules.

The stimulatory influence of the irradiated pollen as evidenced from the results obtained on M_1 seedling height, number of grain-bearing tillers, mature plant heights, panicle lengths and to a lesser degree, on seed weights, collaborate with the findings of Rudolph (1965; 1969) who reported that in *Picea glauca* (white spruce) the stimulatory effect of pollen irradiation on seed germination, seed yield, seed set and viability was more evident at lower pollen exposures. Likewise, Clausen (1973a) reported a possible stimulatory effect of pollen irradiation on the rate and percentage of germination in *Betulla nigra* at low levels of radiation, a dose of 500 r to be effective but differences were not statistically significant. Rudolph (1965) explained these observations to be at least partially due to reduction in zygotic and embryo competition resulting from radiation-induced lethals in a portion of the pollen population exposed to low dosages. He also attributed increase in seed weight to an increase in embryo size.

Low doses of radiation may disturb the activity of certain enzymes involved in the synthesis of growth hormones, probably gibberellins. Generally, gibberellic acid 3 (GA_3) was reported to promote cell elongation by increasing the plasticity of the cell walls and by promoting the growth of the radical in the germination of seeds (Edmond, *et al.*, 1978).

Casarett, 1972 mentioned that radiation may, under certain conditions, result in an increase in physiologically active substances, possibly by destruction of inhibitory substances. The controversial growth stimulation effect might be attributed to such a change.

Inability of the seed to germinate may be due to permanent inhibition on mitosis or due to death of cells. Impairment of seedling development may be attributed partly to the production of dominant mutations. Reduction in seedling height and plant height may be due to the temporary suspension of cell division or death of some cells. It is possible that one of the chief causes of cell death in tissues at high doses are chromosome aberrations. Another possible primary cause of cell death could be the effect of ionizing radiation in rendering DNA molecules incapable of serving as a template for the production of normal messenger RNA. The end result is that some enzymes and other proteins cannot be formed and the cell can no longer maintain its normal capacity for sustained cell division. It undergoes a "reproductive death".

The highest pollen exposures resulted in the least growth of seedlings. Casarett (1972) mentioned of a reversal of the seedling height curve at high exposures; that is, for low doses there is a progressive decrease in seedling heights with increasing exposures. With high doses, however, there is a slight reversal or apparent increase in leaf length relative to that at a slightly lower exposure. This reversal has been explained as the result of interaction between chromosome damage and mitotic inhibition. Extensive chromosome damage in irradiated dividing cells will result in a high degree of lethality of daughter cells. At higher doses, the chromosome damage is greater, but mitosis has been so completely inhibited that cells do not die in a mitotic-linked death. They survive and are apparently still capable of elongation to produce leaves. The radiation exposure at which the reversal occur differs with species.

Generally, more aberrations are produced at high doses than at low doses. At high doses many chromosomes are broken and many of the broken ends may rejoin or unite in a process called restitution. In such cases, no lesion will be visible in the chromosomes, and most likely, complete recovery will be achieved. If the break does not heal, the end portion will remain as a chromosome fragment.

A low frequency of anaphase bridges and fragments were found in mitotic cells of M_2 plants. It has been found that the frequency of bridges is directly proportional to the dose of radiation as in x-rays (Caldecott, *et al.*, 1954). The occurrence of few bridges probably indicate fusion of broken ends of the majority of weight, seed germination, seedling height, chromosomal aberration, number of grain-bearing tillers, mature plant height and panicle length in M_1; and pollen stainability, seed germination, chromosomal aberration and panicle length in M_2.

M_1 generation

In M_1 there was a significant reduction in pollen stainability with increasing doses of gamma radiation at 1, 5, 7 and 9 Kr gamma rays.

The mean percentage seed set decreased significantly and linearly with increasing radiation dose at 1, 3 and 9 Kr gamma rays.

There was a slight increase in mean seed weights at 3 Kr with 2.30 gm and at 7 Kr with 1.93 gm. The following gamma ray doses were not significantly different: 1 and 3 Kr as well as between 5 and 7 Kr gamma rays.

In germination the following gamma ray doses were significantly different: between control and 1, 3, 5, 7 and 9 Kr.

On seedling height highly significant differences were noted among the treatments: control and 5 Kr, control and 7 Kr, control and 9 Kr, 3 and 5 Kr, 7 and 9 Kr.

Two types of chromosomal aberrations were obtained: bridges and fragments.

A stimulatory effect of pollen irradiation on the number of grain-bearing tillers was greatly expressed after the 3 Kr treatment.

The following gamma ray doses for mature plant height were not significantly different: control, 1, 3, 5 and 7 Kr.

The following gamma ray doses for panicle length were not significantly different: control, 1, 3 and 5 Kr, between control and 7 Kr, between 5 and 7 Kr and between 7 and 9 Kr.

M_2 generation

In M_2 the following gamma ray doses were significantly different in pollen stainability: between control and 5, 7 and 9 Kr, between 1 and 5, 7 and 9 Kr and between 3 and 5, 7, 9 Kr.

The following gamma ray doses for seed germination were not significantly different: control, 1, 3 and 5 Kr and between 7 and 9 Kr treatments.

The following gamma ray doses did not differ significantly in producing chromosomal aberrations: 1, 3, 5 , 7 and 9 Kr.

The following gamma ray doses did not differ significantly with each other in panicle length: between 1, 5 and 9 Kr and between 3, 7 and 9 Kr.

Four types of chlorophyll deficient seedlings were obtained: *albina, chlorina, virescent* and *striata.*

A gradual increase in chlorophyll mutation frequency was noted with increasin gamma ray doses up to 7 Kr but a decrease in chlorophyll mutation rate was observed at 9 Kr.

Based on the results of this study, the following conclusions can be made: (1) Gamma irradiation of pollen grains is effective in inducing genetic variability in rice; (2) Frequency of chromosomal aberrations and chlorophyll mutation rates appear to be associated with induced morphological and other mutations; (3) LD_{50} need not be attained to get the maximum number of mutations; and (4) The ideal dose which gives high mutation rates in rice appears to be at 7 Kr pollen exposure.

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